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ASSESSMENT OF THE PHENOLOGICAL AND AMPELOGRAPHIC VARIABILITY OF GRAPEVINE GENETIC RESOURCES

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ABSTRACT

The study evaluated the phenological and morphological diversity of several varieties from the germplasm collection at INCDBH Ștefănești. We analyzed phenology between 2023 and 2025 using the BBCH scale, while assessing morphological traits based on 41 OIV descriptors. Statistical analyses included PCA and hierarchical clustering. Phenological stage durations ranged from 153.0 to 159.2 days, with significant annual variation. The first two PCA components accounted for 70.34 % of the phenological variability, and 68.3 % of the OIV descriptors exhibited variability. The results highlight the value of this collection for the conservation of genetic resources and for grapevine breeding.

INTRODUCTION

The grapevine (*Vitis vinifera* L.) is a perennial horticultural species whose development and berry quality are strongly influenced by climatic conditions. Temperature, precipitation, solar radiation, and water availability affect the timing and duration of phenological phases, as well as the accumulation of sugars, organic acids, and phenolic compounds. Consequently, grapevine phenology serves as an important indicator of the plant's responses to climatic variability and genotype-environment interactions (Droulia and Charalampopoulos, 2022; Cameron et al., 2022). Climate warming has influenced grapevine phenology in European wine-growing regions, although responses vary depending on the genotype, geographical location, and climate conditions. In Romania, studies have highlighted the influence of climatic factors on grapevine development, berry composition, and regional viticultural suitability (Bucur et al., 2012; Bucur and Dejeu, 2016; Bucur et al., 2019; Tăbaranu et al., 2024). Previous research on grapevines and other perennial horticultural species has also underscored the importance of evaluating phenological responses in relation to climatic variability and genotype (Florea et al., 2020; Dumitru et al., 2023; Manolescu et al., 2026).

In addition to the study of phenology, morphological characterization is essential for assessing the genetic diversity of grapevines. Standardized OIV descriptors facilitate the identification and differentiation of accessions, particularly local and traditional genotypes (old/traditional varieties) that may represent valuable genetic resources. In this context, Ilina (Dumitru) et al. (2023b) analyzed the morphological diversity of grapevine accessions in the collection at Ștefanesti, highlighting the relevance of ampelographic characterization for the assessment of germplasm. Consequently, a combined assessment of phenological and morphological traits can provide a deeper understanding of grapevine diversity and its potential for conservation, breeding, and adaptation to changing climatic conditions.

In this context, the present study aimed to characterize grapevine genotypes maintained in the INCDBH Stefanesti germplasm collection by evaluating phenological variability (over 2023–2025) and morphological diversity (based on OIV descriptors) of grape berries. Univariate and multivariate statistical analyses were used to characterize variation among genotypes and growing seasons, as well as to establish phenological and ampelographic characteristics and the utilization of grapevine genetic resources in breeding programs focused on enhancing resilience to changing climatic conditions.

MATERIAL AND METHODS

The study was conducted during 2023–2025 in the grapevine germplasm collection of the National Research and Development Institute for Biotechnology in Horticulture, Stefanesti-Arges, Romania.

Five grapevine genotypes were evaluated, including the white-berried ‘Putna’ and ‘Olesia’ and the black-berried ‘Muscat de Hamburg’, ‘Tâța caprei neagră’ and ‘Transilvania’.

Phenological observations were conducted from budburst to grape ripening using the BBCH scale (Coombe, 1995; Maier, 2001). The dates of occurrence and duration of the main phenological stages were recorded for each genotype and year. Air temperature and precipitation during the growing season were monitored using an automatic IMETOS weather station. Morphological characterization was performed using 41 OIV descriptors according to the *OIV Descriptor List for Grapevine Varieties and Vitis Species*.

The variation among genotypes in relation to the variables analysed was investigated using principal component analysis (PCA) on standardized data. PCA was used to reduce dataset dimensionality and identify the main sources of variability. The contribution of each principal component was expressed as the percentage of total variance explained.

Hierarchical cluster analysis (HCA) was used to assess the degree of similarity among genotypes, with the results presented as dendrograms. For the phenological analysis, genotypes were clustered based on the duration of the main BBCH phenological stages evaluated over the 2023–2025 period. For morphological characterization, the analysis was based on variable OIV descriptors, while invariant descriptors were excluded because they did not contribute to genotype differentiation.

Multivariate analyses enabled the identification of similarity relations among genotypes and the determination of the variables that contributed most strongly to the differentiation of the biological material under study.

RESULTS AND DISCUSSIONS

The results show interannual variations in the duration of the main phenophases, while the mean cumulative duration of the phenological cycle showed smaller fluctuations. Annual mean values ranged from 153.0 days in 2025 to 159.2 days in 2024, with a difference of 6.2 days between the extremes. In contrast, the amplitude of variation differed among phenophases, suggesting that interannual variability was shown mainly in the temporal distribution of successive developmental stages.

Table 1.

Mean period of the main phenological stages in grapevine genotypes studied during 2023–2025

Phenological stage	BBCH	2023 (days)	2024 (days)	2025 (days)
Sprouting/Bud development	00–09	15.0 ± 1.87	15.0 ± 2.55	17.8 ± 2.86
Leaf development	11–19	15.8 ± 2.28	15.8 ± 1.92	25.4 ± 3.13
Inflorescence emerge	53–57	17.6 ± 2.51	19.0 ± 2.55	15.2 ± 2.59
Flowering	60–69	22.8 ± 2.77	18.0 ± 2.24	15.4 ± 1.67
Development of fruits	71–79	49.8 ± 4.02	47.8 ± 1.48	41.6 ± 5.13
Ripening of berries	81–89	37.4 ± 2.41	43.6 ± 2.70	37.6 ± 4.56
Total period of the phenological cycle	—	158.4	159.2	153.0

**Values are expressed as mean ± standard deviation (SD), calculated for the five genotypes analysed in each year.*

The most significant change was recorded in grapevine leaf development (BBCH 11–19). The mean duration of this phenophase was 15.8 days in both 2023 and 2024, increasing to 25.4 days in 2025. This represents an increase of approximately 9.6 days, or more than 60 %, compared to the previous two years. This trend indicates slower early vegetative development in 2025.

Meanwhile, the flowering and grape development stages tended to occur over shorter periods in 2025. The mean period of flowering (BBCH 60–69) decreased progressively from 22.8 days in 2023 to 18.0 days in 2024 and 15.4 days in 2025. Thus, between 2023 and 2025, the period of this phenophase decreased by 7.4 days, corresponding to approximately 32.5 %.

A similar pattern was observed for grapevines fruit development (BBCH 71–79). This stage had the longest mean period among the phenophases analysed; however, the duration decreased from 49.8 days in 2023 to 47.8 days in 2024 and 41.6 days in 2025. Compared with 2023, grapevines' fruit development was therefore 8.2 days shorter in 2025.

The interannual variation observed in the main phenophases is similar to previous research conducted on the genetic material preserved in the grapevine germplasm collection at Ștefănești. Studies conducted by Ilina (Dumitru) et al. (2023) on several grapevine accessions preserved in the germplasm collection at Ștefanesti have shown that changing climatic conditions can lead to shifts in phenological development, with budburst occurring approximately 5–14 days earlier and the onset of flowering 8–23 days earlier. The results of the present study show that interannual variability may influence not only the calendar timing of phenophase onset but also the duration and relative distribution of successive developmental stages.

A different pattern was observed for ripening (BBCH 81–89). The mean period of this phenophase was 37.4 days in 2023, increased to 43.6 days in 2024, and subsequently decreased to 37.6 days in 2025. Thus, 2024 was characterised by a ripening period approximately six days longer than those recorded in the other two years. This indicates that changes occurring during the early vegetative stages do not necessarily persist in the same direction throughout grape maturation.

The individual genotype analysis also revealed differential responses. In 2025, grape development period ranged from 33 days in 'Putna' to 46 days in 'Olesia', while ripening period varied from 33 days in 'Putna' to 43 days in 'Transilvania'. In 2024, fruit development was more uniform across genotypes, with values ranging from 46 to 50 days.

Genotypic differences were also evident in the mean cumulative period of the grapevine phenophases over the three-year study period. 'Putna' showed the shortest mean phenological cycle, at approximately 149.0 days, followed by 'Muscat de Hamburg' (155.3 days) and 'Olesia' (158.7 days). Furthermore, 'Transilvania' (160.0 days) and 'Tâța caprei neagră'(161.3 days) recorded the longest mean durations. These differences highlight genotype-specific characteristics in the rate at which successive developmental stages are completed.

Overall, the study carried out during 2023–2025 revealed considerable phenological plasticity in the genetic material analyzed. Relatively small variations in the total duration of the phenological cycle occurred alongside more marked changes in the duration of individual phenophases, particularly the extension of leaf development and the shortening of flowering and fruit development in 2025. These results highlight the importance of assessing the main BBCH phenophases separately and support the use of multivariate methods to identify patterns of variation among genotypes and determine the main sources of phenological variability.

Multivariate Analysis of Phenological Variability

Principal component analysis (PCA) revealed a clear pattern in the structuring of phenological variability according to the study year and the characteristics of the genotypes analysed (Figure 1). The first two principal components explained 70.34 % of the total variance, with 50.39 % attributed to the first principal component (PC1) and 19.95% to the second (PC2). These results indicate that the structure of the phenological data was represented in a two-dimensional space.

The first principal component (PC1) was primarily associated with fruit development period, which showed a positive contribution, whereas grapevine leaf development and budburst were oriented in the opposite direction. This distribution highlights a contrast between the prolongation of early vegetative development and the period of subsequent fruit development. The contrast of these variables along PC1 suggests that observations characterised by a longer leaf development period were generally associated with a shorter fruit development period.

The PCA showed separation of the 2025 observations from those recorded in 2023 and 2024. Most observations corresponding to 2025 were located on the negative side of PC1, close to the vectors associated with grapevine leaf development and budburst. This pattern is significant in the univariate analysis, which revealed a substantial increase in the period of the BBCH 11–19 stage in 2025, accompanied by a reduction in fruit development period.

The second principal component (PC2) primarily differentiated observations according to the period of ripening and flowering, with the corresponding vectors oriented

in opposite directions. This relationship suggests contrasting patterns between the flowering phase and the final stages of fruit maturation, contributing to further differentiation among genotype × year combinations within the same growing season.

Furthermore, the dispersion of points corresponding to the same year indicates that phenological responses were not uniform across all genotypes. For example, 'Putna' in 2025 occupied a position farther from the other observations recorded in the same year, reflecting a distinct phenological profile, characterised by a prolonged budburst period and comparatively short periods of fruit development and ripening. This differentiation confirms the contribution of genotype-specific characteristics to phenological responses, although the interannual effect was dominant for several of the phenophases analysed.

Overall, the PCA results support the individual phenophase analyses and indicate that 2025 shows the most distinct phenological profile among the three growing seasons analyzed. This separation cannot be attributed to a single phenophase but rather to simultaneous changes in the timing of several developmental stages, particularly grapevine leaf development, flowering, and fruit development.

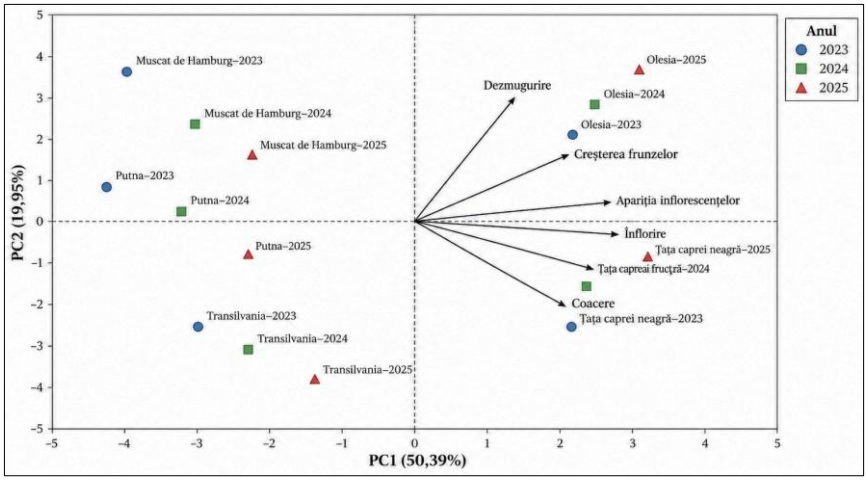


Figure 1. Principal component analysis (PCA) of phenophase duration variability in five grapevine genotypes during the 2023–2025 period

Hierarchical clustering analysis supported the PCA results by highlighting the degree of similarity among grapevine genotypes based on the phenological profiles recorded during the 2023–2025 period (Figure 2). The dendrogram structure revealed the separation of genotypes into distinct groups, based on similarities in the duration of the main BBCH phenophases.

The grapevine genotypes 'Olesia' and 'Tâța caprei neagră' exhibited the greatest similarity in terms of their mean phenological profiles, clustering at a low dissimilarity level. 'Transilvania' genotype joined this cluster, indicating relatively similar phenological behaviour among the three genotypes during the study period. Their similarity resulted from the combined duration of the phenophases rather than from a single developmental stage, confirming the usefulness of multivariate analysis for characterising phenological behaviour.

In contrast, the ‘Putna’ genotype exhibited a more distinct phenological profile. This position may be primarily attributed to the shorter overall duration of the phenophases analysed, with a three-year mean of approximately 149 days, compared with 158.7 days for ’Olesia’, 160.0 days for ’Transilvania’, and 161.3 days for ’Tâța caprei neagră’ genotypes. In 2025, the distinctiveness of ’Putna’ was further accentuated by the shorter duration of fruit development and ripening, both phenophases lasting 33 days.

’Muscat de Hamburg’ genotype also exhibited a distinct profile compared with the cluster including ’Olesia’, ’Tâța caprei neagră’, and ’Transilvania’. The specificity was associated with a different distribution of phenophase durations throughout the annual developmental cycle. For example, in 2023, fruit development lasted 56 days, representing the highest value recorded for this phenophase in the dataset.

The differences observed among genotypes in terms of phenological development are consistent with previous results for the genetic material preserved in the grapevine germplasm collection at Ștefănești. Ilina (Dumitru) et al. (2023a) highlighted considerable variability in the main phenophases among the grapevine accessions analyzed, with their responses depending on both genotypic characteristics and climatic conditions. The researchers reported shifts in the timing of budburst and flowering, confirming the high sensitivity of grapevine phenological development to variability in environmental conditions.

Comparing the HCA and PCA results highlights two complementary aspects of phenological variability. PCA primarily revealed interannual differentiation, with 2025 exhibiting the most distinct profile, whereas hierarchical clustering identified similarities among genotypes based on their overall phenological stages.

Overall, the results of both multivariate analyses indicate that the phenological variability of the studied plant material results from the combined effects of genotypic characteristics and interannual variability. Clustering genotypes according to phenophase duration therefore provides additional information beyond the individual analysis of each phenophase and enables the identification of distinct phenological patterns within the germplasm collection.

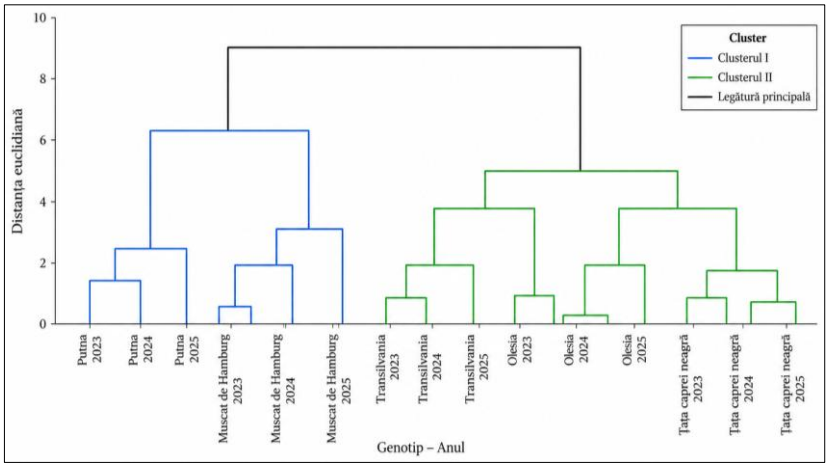


Figure 2. Dendrogram of phenological similarity among the five grapevine genotypes based on phenophase duration during the 2023–2025 period

Morphological Variability Based on OIV Descriptors

Ampelographic characterisation revealed notable morphological variability among the five genotypes analysed. Of the 41 OIV descriptors used for evaluation, 28 (68.3 %) exhibited variation, whereas 13 descriptors (31.7 %) showed similar values across all studied accessions. The high percentage of variable descriptors indicates a high level of morphological diversity within the genetic material studied.

The descriptors that showed no variation among the five genotypes were OIV 001, OIV 003, OIV 007, OIV 008, OIV 068, OIV 81-2, OIV 083-2, OIV 151, OIV 235, OIV 241, OIV 351, OIV 505, and OIV 506. The descriptors represent traits shared by all five genotypes and therefore have limited discriminatory power within the studied group. In contrast, the variable descriptors highlighted differences among accessions and were used in the multivariate analysis of morphological similarity.

A wide range of variation was observed for several descriptors. For example, OIV 004 ranged from 3 to 7, as did OIV 065, while OIV 202 showed values ranging from 5 to 9. Differences were also observed for OIV 220, OIV 221, OIV 223, OIV 301, OIV 303, OIV 502, OIV 503, and OIV 504, indicating that differences among genotypes were associated with variation across multiple ampelographic traits.

These results correspond to previous research conducted on the grapevine germplasm collection at Ștefănești. Ilina (Dumitru) et al. (2023b) used an extensive set of OIV descriptors for the ampelographic characterisation of accessions newly introduced into the collection and demonstrated significant variability in morphological traits. Similarly, the present study confirms the usefulness of OIV descriptors for identifying discriminative traits and assessing the phenotypic diversity of grapevine genetic resources.

To assess morphological similarity among the five genotypes, hierarchical cluster analysis was performed using the variable OIV descriptors. Excluding invariant descriptors from the similarity analysis allowed the classification to focus on traits with discriminatory value.

The analysis revealed the greatest morphological similarity between the 'Putna' and 'Transilvania' genotypes, which formed the first cluster at the lowest dissimilarity level. A second distinct cluster was formed by 'Muscat de Hamburg' and 'Olesia', while 'Tâța caprei neagră' was also included in this broader cluster, suggesting morphological similarities alongside specific traits that distinguish it from them.

Comparing the morphological and phenological analyses revealed that similarities in morphological traits did not necessarily correspond to similarities in phenological patterns. The phenological analysis showed the strongest similarity between 'Olesia' and 'Tâța caprei neagră' genotypes, whereas the OIV descriptor-based analysis revealed a closer relationship between 'Transilvania' and 'Putna'. These differences indicate that relationships among genotypes vary depending on the traits considered and highlight the complementary value of phenological and ampelographic characterisation.

Overall, the OIV descriptors proved useful for distinguishing among the five genotypes and characterising their morphological profiles. Integrating morphological and phenological data provides a more comprehensive assessment of grapevine diversity and can support the selection of genotypes with desirable traits for breeding and conservation purposes. Further research combining biochemical characteristics is needed to clarify the relationships between morphological traits, phenological development, and grape composition, thereby improving the evaluation and use of grapevine genetic resources.

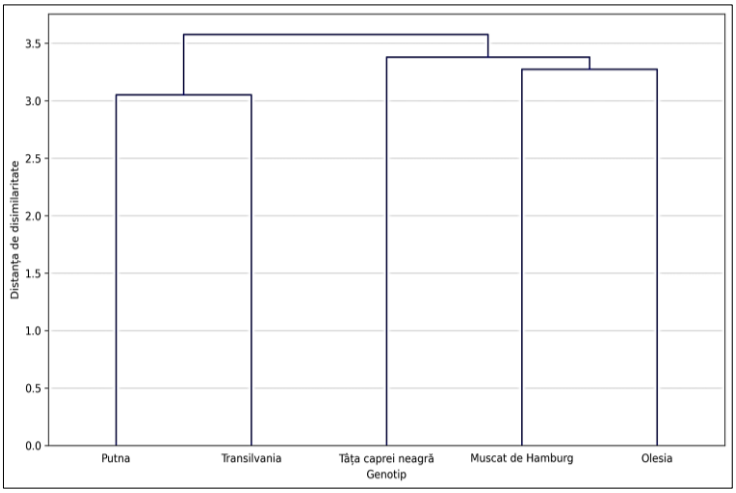


Figure 3. Dendrogram of morphological similarity among the five grapevine genotypes based on 28 variable OIV descriptors

CONCLUSIONS

The study carried out over the 2023–2025 period revealed substantial interannual phenological variability among the grapevine genotypes analysed. The mean cumulative period of the six BBCH phenological stages was 158.4 days in 2023, 159.2 days in 2024, and 153.0 days in 2025, with differences between years being primarily attributable to changes in the duration of individual phenological stages. In 2025, the mean duration of leaf development increased to 25.4 days, compared with 15.8 days in both 2023 and 2024. In contrast, the duration of flowering decreased from 22.8 days in 2023 to 15.4 days in 2025, while fruit development decreased from 49.8 to 41.6 days. The year effect was statistically significant for leaf development ($p < 0.001$), flowering ($p = 0.005$), fruit development ($p = 0.027$), and ripening ($p = 0.009$).

Principal component analysis (PCA) revealed differences in phenological patterns among the three growing seasons, with the first two principal components explaining 70.34 % of the total variance (50.39% for PC1 and 19.95 % for PC2). The 2025 season displayed the most distinct phenological profile, mainly associated with a longer duration of grapevine leaf development and a shorter fruit development period. Ampelographic characterization also revealed diversity among genotypes. Of the 41 OIV descriptors, 28 (68.3 %) exhibited variability, whereas 13 (31.7 %) were invariant. Hierarchical cluster analysis identified distinct groups of morphological similarity, demonstrating the ability of variable OIV descriptors to discriminate among the genetic materials analysed.

Overall, the combined assessment of phenological, ampelographic, and biochemical traits revealed distinct and complementary profiles among the genotypes analyzed. The observed variability highlights the value of the germplasm collection as a source of genetic diversity for the conservation of grapevine genetic resources and the identification of genotypes with useful traits under changing climatic conditions.

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